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A STUDY OF THE SEASONAL CHANGES IN THE
THYROID AND ADRENAL GLANDS OF THE
THIRTEEN-LINED GROUND SQUIRREL (*CI-
TELLUS TRIDECEMPLINEATUS*), WITH PAR-
TICULAR REFERENCE TO ITS SEXUAL CYCLE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1934

By
MOSES ZALESKY

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MOSES ZALESKY

Hull Zoölogical Laboratory, The University of Chicago

THREE TEXT FIGURES AND ONE PLATE (SIX FIGURES)

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INTRODUCTION

Our knowledge concerning the relation of the thyroid gland to sexual activity is at present extremely indefinite, and the experimental analysis of the problem has thus far yielded only disappointingly conflicting results. This lack of agreement regarding the role of the thyroid in the hormonal mechanism underlying sexual activity is apparent from the work done by various investigators on the effects of experimental hypo- and hyperthyroidism upon the reproductive system and is summarized, in tabular form, in chapter XVII

¹ This investigation has been aided by a grant from the Committee on Research in Problems of Sex of the National Research Council; grant administered by Prof. F. R. Lillie.

of 'Sex and Internal Secretions.'² In addition to these data, the conclusion of Evans and Simpson ('30) that thyroid ablation lowers the sex stimulating capacity of the anterior lobe of the hypophysis disagrees with the findings of Smith and Engle ('30) which indicate that thyroidectomy does not affect the gonadotropic function of the anterior pituitary. Van Horn ('33) confirms the observations of Smith and Engle and suggests that, at least in the female albino rat, the thyroid may influence the reproductive system by regulating, through metabolic channels, the rate of elimination of oestrin from the organism. Smelser ('34) finds that thyroidectomized male rats have smaller reproductive accessories and testes with fewer sperm, but that the gonad stimulating potency of their pituitaries seems to remain unaltered as compared with pituitaries of unoperated controls.

The confusion evidenced by these and by many other disharmonious results, calls for a more thorough investigation of the fundamental aspect of the problem, namely, of the reaction of the thyroid gland to natural fluctuations in sexual activity. It was realized, however, that ordinary laboratory rodents could not be used for this purpose since, in these species, the males are continuously capable of breeding throughout the sexually active age, and the females have a short oestrous cycle. Thus, sexual periodicity in the female albino rat is obviously too short cycled to induce readily demonstrable variations in the weight or histological appearance of its thyroid gland. The choice of a mammal with an annual reproductive cycle seemed, therefore, to be particularly desirable for the present investigation, and the thirteen-lined ground squirrel (*Citellus tridecemlineatus*) offered the additional advantages of convenient size, satisfactory tolerance of captivity, and relatively frequent occurrence in the Chicago region. Accordingly, the seasonal variations in the thyroid gland of this animal constitute the chief object of the present study, while the experimental work included here will serve, it is hoped, to throw some light upon the factors responsible

² A contribution of Severinghaus, Engle and Smith to this admirable volume; listed in 'Literature Cited' under the authors' names.

for such thyroid periodicity as may be found to exist in the species under investigation.

It should be mentioned in this connection that the work reported here represents one unit in a cooperative research-project on the physiology of reproduction in relation to the endocrine glands. A paper summarizing the general results of this project appeared elsewhere (Moore, Simmons, Wells, Zalesky and Nelson, '34).

It is the writer's pleasure to acknowledge his indebtedness to Prof. C. R. Moore, the initiator and supervisor of the project, for his generous aid and instructive guidance throughout the course of this investigation; to Dr. Geo. F. Simmons and Dr. L. J. Wells, who studied the sexual aspect of the general problem in females and males, respectively, of *Citellus tridecemlineatus*—for furnishing most of the animals used in the present investigation as well as for supplying the writer with expert diagnoses of the sexual condition of these animals; to Prof. R. R. Bensley and Dr. W. O. Nelson, for their helpful suggestions regarding certain technical procedures employed here; and to the chemists who kindly furnished the various hormone preparations utilized, namely, to the members of the department of physiological chemistry and pharmacology of The University of Chicago, Dr. H. B. Van Dyke and Mrs. Z. Wallen-Lawrence, for pituitary hebin, and Dr. T. F. Gallagher and Prof. F. C. Koch, for testis hormone; to Dr. R. G. Gustavson of the University of Denver, for oestrin; and to Dr. Oliver Kamm of Parke, Davis & Co., for Antuitrin S and whole sheep pituitary suspension, supplied through the courtesy of Dr. W. O. Nelson. The raw human pregnancy urine used in this investigation was obtained from local sources.

MATERIAL AND METHODS

Most of the glands treated in this study were removed at autopsy from animals used by Dr. Geo. F. Simmons and Dr. L. J. Wells in their investigation of the sexual cycle in *Citellus tridecemlineatus*.

The fixing agents employed were Bouin's fluid and preferably Bensley's formalin-Zenker.³ Paraffin sections were obtained 4 to 5 μ in thickness. The staining was done chiefly with Krichesky's ('31) modification of the Mallory connective tissue stain; hematoxylin (Harris' modification), counter-stained by eosin, was also used.

THE HISTOLOGICAL APPEARANCE OF THE THYROID GLAND DURING THE HIBERNATION PERIOD

The most conspicuous features of the thyroid of the male and female ground squirrel during the hibernation period (November to end of March) are the abundance of stored colloid within the follicles and the low epithelium of the follicular wall. The acini are well rounded in outline, apparently distended by the great masses of colloid contained within them.

The typical winter thyroid of animals either dug up from outdoor hibernating pens and found in various degrees of torpor, or of animals taken from laboratory confinement, is essentially a 'resting' gland (fig. 3). Each cell of its squamous follicular epithelium contains a greatly compressed nucleus which appears elliptical in section. The scanty cytoplasm is homogeneously and finely granulated, but does not contain inclusions suggestive of storing, secretory, or colloid resorptive activity. The colloid within the follicle is dense and stains a brilliant pink with eosin and intense blue or deep red with Mallory. In Mallory stained preparations, the colloid within certain follicles may appear red in the center and blue at the periphery of the lumen. Occasionally a still finer differentiation is encountered: adjoining the inner surface of the follicular wall, there may be a varyingly wide, often vacuolated border of secretion staining a pale blue⁴ and gradually blending into the dark blue, or, through the

³ The stock solution contains 2.5 gm. of potassium bichromate and 5.0 gm. of mercuric chloride per 100 cc. of distilled water; for the fixative, one part of pure, neutral formalin is added to nine parts of the stock solution.

⁴ Henceforward, throughout this report, all references to the staining reactions of the colloid will be made on the basis of Mallory-stained preparations.

dark blue, into the deep red of the central colloid. Whether, however, the red-blue differentiation of the colloid can serve as a criterion of physiological activity or of comparative freshness of thyroid secretions, is still open to question. Uhlenhuth ('27), on the basis of his studies on the salamander thyroid, maintains that the colloid staining red with Mallory is basophilic while that which takes the deep blue stain is acidophilic, and that the former is staler than the latter. These deductions, however, are far from representing established facts. The writer, from his experience, has ascertained that, in the matter of the red-blue differentiation in the colloid, one is dealing with a great many variables; chief among these are the fixing agent employed (thus, bichromate fixatives tend to produce in the colloid a more pronounced affinity for the red) and differences in the length of fixation time or in the comparative duration of exposure to the Mallory I solution (acid fuchsin) and to the triple Mallory II mixture (a longer immersion in the latter invariably tends to stain the colloid blue). A rigid technical constancy in preservation of tissue for histological purposes is humanly impossible, and one cannot be too certain that even the slightest deviation from routine will not cause appreciable differences in so fickle a matter as the red-blue differentiation of the thyroid colloid.

The typical picture of the winter thyroid described above is not exclusively met with during the period under discussion. In some instances, winter glands are composed largely or wholly of follicles with an epithelium ranging between low cuboidal and cuboidal and consisting of cells actively engaged in the process of storing colloid. The apical portion of a cell of this type contains abundant colloid or colloid-like floccules staining the same shade of blue as the intrafollicular colloid and apparently representing material ready for storage (figs. 1 and 4). The nucleus is large and round, and lies close to the basal pole of the cell.

Uniformity of structure and physiological condition in all follicles of a given thyroid is seldom encountered. This is true of glands obtained during the winter as well as of those

taken during the spring, summer and early autumn. Each follicle exhibits a certain degree of physiological independence with regard to the remaining acini of the same gland, and an estimate, from histological appearance, of the grade of activity in a given thyroid must be based upon the condition found in the majority of its follicles. The peripheral follicles, as a rule, are larger, often to a considerable extent, than the acini centrally located within the gland. They also possess a lower epithelium and contain denser colloid than the central alveoli. Some of these latter, in winter glands, often present a striking contrast to the large, apparently sluggish peripheral follicles: their epithelium is higher, namely, low cuboidal or cuboidal, and their lumina are filled with colloid staining pale blue and containing numerous fine, practically unstainable bubbles; this variety of colloid is more characteristic of April to October glands. Aron ('30) describes a similar type of colloid in thyroids of young guinea pigs injected for extended periods of time with anterior pituitary extracts. He regards such colloid as a fresh product of resumed storing activity ('néo-sécrétion') following colloid exhaustion. That, also in the ground squirrel, this type of colloid represents freshly stored secretion, is suggested by the fact that the epithelial cells of the follicles in question usually contain in their apical portion fine, blue staining colloid globules indicating that storing activity is in progress. What, however, becomes of this apparently thin intrafollicular colloid if not discharged too soon into the blood stream? Does it condense, through the mere physical effect of accumulation and increase in concentration, into the deep staining colloid characteristic of the winter thyroid? Does it do so by undergoing certain chemical changes? Or does it remain unchanged, distinct physically and perhaps chemically from the dense, homogeneous colloid typical of the winter gland? The answer to these questions is still within the realm of pure conjecture. Nor are we prepared to say whether or not the colloid under discussion represents unripe secretion, as its chemical composition and comparative thyreoglobulin content have not been determined.

Striking differences also exist in the amount of vacuolization of the intrafollicular colloid in the various alveoli of the same gland. Particularly well vacuolated is the colloid of the large peripheral follicles, as well as that of some central follicles containing the lighter staining colloid referred to above. The physiological significance of this rather conspicu-

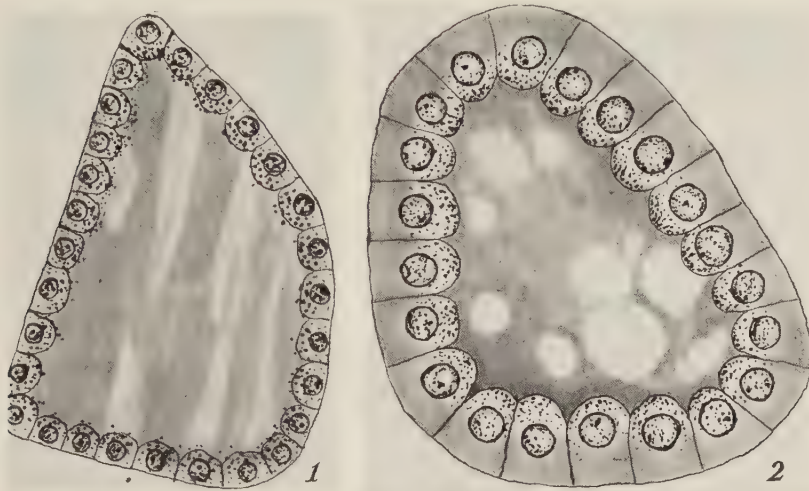


Fig. 1 A camera lucida drawing of a 'storing' winter follicle from the gland represented in figure 4, at a somewhat higher magnification, to show the apical colloid floccules in the epithelial cells.

Fig. 2 A follicle from a summer thyroid (of male no. 712, sacrificed on July 20, 1933) which exhibits reversed polarity. Note the apical position of the nuclei and the accumulation of colloid in the basal portion of the epithelial cells. Formalin-Zenker; Krichesky-Mallory. (Drawn with aid of camera lucida.)

ous distinction is, however, doubtful. If we should agree with Uhlenhuth that certain types of these vacuoles represent chromophobic secretion, we would be unable to account for the presence and frequent abundance of these very vacuoles in the expanded peripheral follicles whose extreme inactivity is evident. Nor does Aron's interpretation of the vacuoles in question as 'vacuoles de résorption,' i.e., as structures associated with the process of colloid exhaustion, fit in with

the fact just cited: active resorption is invariably correlated with a heightened epithelium. It is most probable that vacuoles contained within the intrafollicular colloid are largely, if not wholly, artifacts. In several cases, the writer fixed one-half of the thyroid in Bouin's fluid while the other half of the same gland was fixed in formalin-Zenker. Microscopic preparations made from the Bouin fixed material invariably contained a strikingly greater number of intrafollicular colloid vacuoles than sections obtained from the portion of the gland fixed in Zenker-formol. Even in unfixed, freshly removed material, where these vacuoles have been observed by various investigators, they may represent rapid post-mortem changes.

Desquamation of follicular cells into the intrafollicular colloid is rather infrequent in the winter thyroid. Such desquamation is usually associated with a high degree of thyroid activity. Partial or complete destruction of follicles was, however, observed in several instances. The nuclei become pyknotic, the cytoplasm gradually disintegrates, and the colloid is extruded into interfollicular spaces. There it remains for some time, apparently being too dense to be absorbed rapidly by the lymph stream.

A characteristic, conspicuous element in the winter thyroid is the interfollicular cell islet (figs. 3 and 4). Similar islets have been described in the thyroids of other mammals, e.g., man (Marine), rabbit (Biedl). They consist, in *Citellus tridecemlineatus*, of compact masses of large, irregularly shaped and irregularly grouped cells with round, vesicular nuclei. These cells differ from the cells of the follicular epithelium also in their staining reaction. Thus, the cytoplasm of the islet cells takes a bluish tint and appears to be filled with minute blue staining granules, whereas the cytoplasm of the follicular cell stains purplish and lacks corresponding inclusions. That these interfollicular cell nests constitute potential follicles, there can be no doubt; the writer has had the occasion to observe various stages of their transformation into acini. This process will be described in the discussion of the spring thyroid where it is of more common occurrence.

Thus we see that the typical thyroid of either the male or the female ground squirrel during the hibernation period shows follicles with a low, inactive or little active epithelium and with a dense variety of colloid stored to capacity and apparently ready to be mobilized on demand for the needs of the organism. Follicles or entire glands actively engaged in storing colloid may also be encountered. It is significant to note that the same low grade of activity prevails in thyroids of laboratory-confined adult males sacrificed in January or February and whose reproductive system, at that time of the year, approaches or attains the breeding condition.⁵

THE HISTOLOGICAL PICTURE IN THE THYROID GLAND DURING THE NON-HIBERNATING SEASON (APRIL TO OCTOBER)

The thyroid of both the male and the female ground squirrel, shortly following the animal's emergence from hibernation, does not differ markedly from the winter gland described above. Soon, however, about the middle of April, changes indicative of heightened activity become evident. These changes appear first in the follicles centrally located within the gland and proceed gradually toward its periphery, reaching their spring climax late in April or sometime during May, in males, as a rule, earlier than in females. The gland as a whole becomes more intensely vascularized, and a high degree of hyperaemia often ensues. The follicular epithelium gains in height, becoming cuboidal, high cuboidal, or columnar. To obtain an indication as to whether this increase in epithelial height represents an actual hypertrophy of the cell or merely the effect of crowding of cells as a result of their proliferation, the following method was employed. Glands from animals representative each of a different season of the year were chosen for comparison. This sampling of thyroids was made on the basis of the greatest epithelial height for every season considered. Fifty follicles were then selected

⁵ For a condensed description of the annual sexual cycle in the male and in the female *Citellus tridecemlineatus*, the reader is referred to the report on the general project (Moore, Simmons, Wells, Zalesky and Nelson, '34).

from central sections of each of these thyroids. It was endeavored to represent in this selection the various types of alveoli as to size, shape, epithelial height, etc., found in the gland. Follicles in tangential section, in so far as they could be recognized, were avoided. An outline drawing was made on paper of the epithelial wall of each selected follicle by means of a camera lucida of the Abbé type, and the number of cells contained in this epithelial wall was noted. The fifty follicles selected from a given gland were divided into five groups of ten, and in each group the total net area of magnified epithelium appearing in the drawing was measured by means of a Keuffel-Esser planimeter. The number of planimeter units contained in this area was divided by the total number of cells present in the ten corresponding acini, and the area per cell in the given group of ten follicles thus obtained. Each group of ten follicles was treated in an identical manner, and the numbers of planimeter units of area per cell in all five groups of follicles of the same thyroid were found to agree very closely (table 1). The mean area per cell for each gland was computed and taken as a basis of comparison between the thyroids chosen, and, since only relative determinations were sought, it remained unnecessary to convert the planimeter units into metric units of area or to calculate the actual measurements from the magnification employed in the process. Hypertrophy of the cell could thus be easily detected while a comparison of the total number of cells in the fifty follicles selected from each thyroid served to suggest the presence or absence of hyperplastic tendencies.

Some characteristic data obtained by this method are given in table 1.

These data demonstrate beyond doubt that the increase in epithelial height which becomes evident in the spring thyroid is largely due to the hypertrophy of the epithelial cell, but that this hypertrophy does not necessarily coincide, either in the male or in the female, with the annual peak of sexual development and activity. In the thyroid of males with scrotal testes of maximal size and with quantities of motile spermato-

TABLE 1

Seasonal and experimentally induced variations in the volume of the epithelial cell in the thyroid of *Citellus tridecemlineatus*, as indicated by measurements of net epithelial area per cell in section

	UNTREATED ANIMALS ¹	EXPERI- MENTAL ANIMALS	DATE SACRI- FICED	SEXUAL CONDITION	GENERAL ESTIMATE OF THYROID ACTIVITY	THYROID EPITHELIUM	NET EPI- THELIAL AREA IN FIFTY FOLLICLES (PLANI- METER UNITS)	NUMBER OF CELLS IN THE FIFTY FOLLICLES MEASURED	RANGE OF EPI- THELIAL AREA PER CELL (PLANIMETER UNITS)	MEAN EPI- THELIAL AREA PER CELL (PLANIMETER UNITS)
Males	741		12/ 3/33	Low	'Storing'	Cuboidal	4148	1446	2.67-3.06	2.87
	454		1/27/33	Advanced; sperm in epididymis	Very low, 'resting'	Squamous	3345	1369	2.40-2.51	2.44
	80		4/ 1/32	At or very near breeding condition	Very low, 'resting'	Squamous	3255	1380	2.30-2.40	2.36
	619		4/21/32	At or very near breeding condition	High	Columnar	6600	1488	4.35-4.48	4.43
	712		7/20/33	Low, regressed	High	Columnar	6286	1438	4.30-4.47	4.37
	772		9/28/34	Very low, completely regressed	High	High cuboidal	3571	1132	2.90-3.41	3.15
		53 ²	12/30/31	Highly stimulated	Very high	Columnar	7796	1470	5.17-5.53	5.30
	687		12/ 3/33	Low	Very low, 'resting'	Squamous	2646	980	2.65-2.77	2.70
	121		4/29/32	At or very near oestrus	Very low, 'resting'	Squamous	2650	976	2.57-2.90	2.71
	161		5/29/32	Pregnant, near term	High	High cuboidal	3478	1160	2.90-3.21	2.99
Females	869		7/12/34	Regressing, lactation just ending	High	High cuboidal	3359	1073	3.09-3.20	3.12
	966		9/14/34	Low, regressed	High	High cuboidal	3112	978	3.03-3.33	3.18
		43 ³	1/ 5/32	Highly stimulated	Very high	Columnar	5587	1342	3.98-4.34	4.16

¹ All untreated animals used for these determinations were normal adults taken from the field or from hibernating pens.

² Injected with pituitary hebin, 1½ cc. daily, for 13 days.

³ Injected with pituitary hebin, 1½ cc. daily, for 20 days.

zoa in the epididymis, and of females in full oestrus, no noteworthy deviation from typical winter conditions was observed. Only toward the end of April or sometime during May, i.e., with the inception of sexual regression in the male, does epithelial hypertrophy manifest itself in its thyroid. In the female, a corresponding increase in the volume of the thyroid epithelial cell, accompanied by marked and often severe hyperplasia in the gland, occurs throughout mid and late pregnancy, i.e., during May (fig. 5), and, to a somewhat lesser degree, also during lactation, i.e., in June (fig. 6). Particularly striking is the thyroid hyperplasia of pregnancy. There is an enormous increase in parenchyma which, in some cases, appears pathologically exaggerated and is accompanied by active colloid resorption. Cell proliferation seems to have run riot in these glands (fig. 5). The presence of numerous hyperchromatic and pyknotic nuclei testifies to an equally intensive destruction of cell and follicle. Surprising, however, is the fact that, despite the most painstaking search, mitotic figures have never been found in our histological preparations of pregnancy thyroids.

The data presented in table 1 indicate, moreover, that epithelial hypertrophy persists in the thyroid of the male until October, i.e., throughout the period during which the testicles and dependent accessories undergo progressive involution. Likewise, the epithelium in the thyroid of the female remains hypertrophied, though not as hyperplastic as during pregnancy and lactation, also throughout the July to October period of sexual regression.

Associated with the epithelial hypertrophy occurring in the thyroid of both male and female ground squirrels during the non-hibernating season, is the appearance, in the cell cytoplasm, of characteristic inclusions which unmistakably suggest a heightened level of activity. Most commonly, the apical portion of the cell contains numerous colloid floccules of varying size. Often, however, these are seen scattered throughout the cytoplasm of the cell, a fact which complicates the interpretation of their physiological significance. Whether

such inclusions, when distributed over the entire cytoplasm of the epithelial cell, represent secretion or secretion antecedents proceeding toward the follicular lumen, or whether they are associated with the resorption of the stored colloid and its transcellular passage in the reverse direction, i.e., from the lumen into the blood channels of the gland, cannot be stated by the critical observer.

A striking peculiarity occasionally encountered in the thyroid of the male *Citellus tridecemlineatus* during the non-hibernating period is the exclusive confinement of intracellular colloid to the basal portion of the epithelial cell. In a thyroid in which this remarkable phenomenon occurs, it involves the overwhelming majority of the follicles of that gland. Apparently, we are dealing here with a distinct phase in the cycle of thyroid activity, a phase whose relatively infrequent occurrence in our material (in approximately 5 per cent of all untreated field males sacrificed during the April to October period) may be accounted for by its presumably brief duration. This phase has never been observed by the writer in untreated females; the fact, however, that it was found in the thyroid of at least two experimental females suggests the possibility that it does occur also normally in animals of that sex and that it would have been encountered had greater numbers of females been sacrificed. In late summer the condition under discussion was met with both in animals born the previous summer as well as in young of the year, a circumstance which excludes age as a factor in the production of this peculiarity.

In preparations obtained from glands fixed in Bouin's fluid, the basal colloid in such epithelial cells is coagulated, retracted, and appears as a vacuolated area containing a reticulum of colloid strands staining deep blue. In formalin-Zenker, however, it is preserved as a homogeneous gel staining pale blue, filling completely and apparently to the exclusion of protoplasm, every corner in the base of the cell, and occasionally extending high toward its apex (fig. 2). The apical portion of the cell in question is invariably free from any

form of colloid and takes the purplish hue of ordinary thyroid cytoplasm; like the latter, it contains fine granules. The cell as a whole increases in volume and becomes columnar or high columnar, and its nucleus is shifted toward the apical end. A similar situation was described by Bensley ('16) in hypertrophied thyroids of the opossum. Bensley's material was fixed in formalin-Zenker and stained with Brasilin-Wasserblau. This author suggests that a cell of the type under consideration has reversed its polarity physiologically as well as anatomically and is secreting through its basal end directly into the blood channels of the glands. He postulates the theory that the thyroid gland normally secretes directly into the blood stream, and that only the excess of produced hormone is stored as colloid within the follicles of the gland; when, however, the demand of the organism for the thyroid hormone becomes so great that it cannot any longer be supplied in sufficient quantities by the direct route, the indirect mode of secretion, namely, the resorption of the stored colloid, is resorted to. It is noteworthy that in *Citellus* thyroids which show polarity reversal and accumulation of colloid in the basal portion of the secretory cell, the intrafollicular colloid, as a rule, is dense and apparently in a non-resorptive state. This fact tends to support Bensley's hypothesis according to which direct secretion of the thyroid hormone precedes the resorption of the stored colloid.

Not only the secretory cell, but also the intrafollicular colloid in the April to October thyroid, as compared with that of the winter gland, undergoes conspicuous changes whose meaning, unfortunately, is not entirely clear. The dense, deeply staining colloid usually disappears from the follicular lumen. It is apparently being diluted by an influx of fresh secretion of a more fluid consistency or liquefied through the agency of certain solvent influences emanating from all or some of the cells of the follicular wall. Most of the intrafollicular colloid now stains pale blue and no longer presents a homogeneous appearance. Thus, it is often speckled by tiny, more or less uniformly distributed droplets of material

chromophobic in tendency and only rarely seen in winter thyroids; in many follicles, and occasionally in entire glands, it appears alveolated, bubbly, a mere 'foam,' so to speak, of colloid (fig. 6). The latter condition is probably indicative of active colloid resorption, a supposition further supported by the fact that the epithelial wall in acini containing such 'foamy' colloid is usually thrown into numerous folds, obviously as a result of follicular collapse accompanying colloid exhaustion.

Dehiscence of follicular cells as well as destruction of follicles are fairly common in thyroids taken during the late spring, summer and early autumn, particularly in glands with a considerable amount of colloid resorption. Occasionally, a cell desquamated into the intrafollicular colloid will show a sound nucleus; usually, however, one finds desquamated cells or groups of cells in various stages of disintegration. In either case, a varyingly wide halo of chromophobic material often surrounds the desquamated cell or group of cells. Apparently, substances released from these cells have produced, within the sphere of their influence, certain physico-chemical changes in the surrounding colloid. Whether these substances simply represent disintegration products or whether they are present in the normal, healthy thyroid cell and, by dissolving the stored colloid, prepare it for resorption, cannot be determined at present.

Most remarkable is the fashion in which interfollicular cell islets are converted into functional follicles. The thyroids taken during the non-hibernating season abound in folliculizing cell islets. One islet may give rise to one, two, three or more follicles. The first follicle to develop in an islet usually arises not in the center of the latter, as might be expected, but somewhere near its periphery. As an early indication of this transformation, colloid spherules appear within the cytoplasm of the cells destined to form the follicle. Gradually a lumen is formed, and the intracellular colloid droplets are discharged into it. The newly formed follicle is, then, eccentric with regard to the islet of its origin, and its wall

consists, on one side, of polarized, definitive follicular cells, and, on the other, of an undifferentiated mass of islet tissue, within which new foci of folliculization gradually arise. Once formed, a follicle increases in circumference by cell proliferation as well as by distension due to accumulation of intrafollicular colloid. Even in a definitive, well-expanded and functional follicle it is not uncommon to find, at one point or another of the follicular wall, a spheroid thickening interrupting the continuity of the epithelium and consisting of undifferentiated cells, obviously a remainder of an interfollicular islet. A 'nest' of this sort may acquire a lumen and become transformed into a diminutive acinus seemingly embedded within the epithelial wall of the main follicle.

In immature animals, the thyroid gland appears to be highly active throughout the first 2 to 3 months of their life. The follicles are relatively small and contain a large amount of parenchymatous tissue and thin, bubbly colloid; the epithelium is high, and its cells show apical colloid floccules; occasionally, in males, polarity reversal and basal colloid are encountered. This hyperactivity in the thyroid of the young animal is evidently a response to the needs of the rapidly growing organism.

On the other hand, the thyroids of laboratory confined adults usually exhibit, during the summer and early autumn, a degree of activity lower than that observed in glands taken from field animals. Such laboratory adults usually fatten, grow inactive, cuddle together in heaps, usually in one of the corners of the cage, and sleep; only occasionally, singly or severally, do they frequent the feeding pan or drinking bowl. Histologically, their thyroids, as a rule, resemble those recovered from winter animals, and are similarly characterized by low epithelium, an abundance of dense, deep staining intrafollicular colloid, little or no colloid resorption, and relatively poor vascularity. Laboratory confined females exhibit a somewhat higher level of thyroid activity than caged males.

SEASONAL FLUCTUATIONS IN THE WEIGHT OF THE THYROID GLAND *

The animals used for this phase of the study and represented in figure A were normal adults taken directly from the field or from outdoor hibernating pens. During January, February and March, however, when outdoor animals were difficultly obtainable, the writer included several laboratory

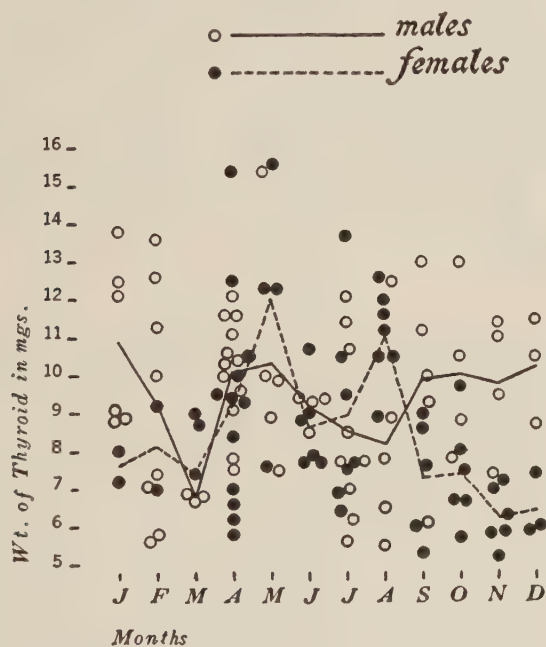


Fig. A Seasonal fluctuations in the weight of the thyroid gland of *Citellus tridecemlineatus*.

confined animals, after having convinced himself that these latter differed in no way, as to thyroid weight and histological structure, from ground squirrels which hibernated in outdoor pens.

The results (fig. A) may be interpreted as follows. Two chief factors influence the weight of the thyroid: the amount of stored colloid and the combined volume of the cellular

*One thyroid from each animal was weighed after fixation in Bouin's fluid, while submerged in the latter.

elements within the gland. Consequently, inactive thyroids with a heavy storage of colloid tend to raise the average thyroid weight during the winter months. On the other hand, the May peak of the curve of seasonal variation in thyroid weight is due to the increase in parenchyma taking place in the gland during that period, and thus represents a true functional peak. It follows, then, that the weight of the thyroid gland cannot be considered a reliable criterion of its activity, a fact which is further emphasized by the wide range of individual variability and by the general irregularity of the curve of seasonal fluctuations in thyroid weight.

The writer feels, therefore, that a comparison of epithelial volume per cell at various times of the year (such as given in table 1) offers a truer index of the seasonal variability in thyroid activity.

EXPERIMENTAL

a. Administration of gonad stimulating preparations

It has been known for some time that administration of anterior pituitary substance, either by subcutaneous injection of gonad stimulating pituitary extracts or by implantation of hypophyseal anterior lobes from certain species, stimulates the thyroid gland to general hypertrophy, increase in height of the thyroid epithelium, and colloid exhaustion (Aron, '30; Grant, '30; Schockaert, '31; Loeb and Friedman, '31 a and b; and others). The laboratory mammal used most extensively and most advantageously in this connection has been the young guinea pig.

The results obtained by the writer in the thyroids of both male and female *Citellus tridecemlineatus* through the administration of gonad stimulating anterior pituitary substance, are in perfect agreement with the observations described in the preceding paragraph. The chief methods of administration of hypophyseal substances in this investigation were: subcutaneous injection of pituitary hebin or of a suspension of whole sheep pituitary powder (Parke, Davis); implantation of rat anterior pituitaries hashed up in a little physiological

saline solution, into the leg muscles of the ground squirrel by means of a large size, wide-shafted hypodermic needle; and implants of guinea pig pituitaries.

The effects of these treatments were particularly striking during the winter when the thyroid gland of the ground squirrel is normally in a resting or storing phase and when there is little or no colloid resorption. By pituitary stimulation one can obtain in December, in either male or female animal, a thyroid which, as regards epithelial hypertrophy and degree of colloid exhaustion, exceeds anything seen in *Citellus* thyroids in May. This fact makes the thirteen-lined ground squirrel an ideal animal for the study of the effects of the anterior pituitary upon the thyroid gland. As a rule, the thyroid of the female displays a considerably lower threshold of irritability with respect to anterior pituitary stimulation than that of the male.

Histologically, the thyroid epithelium increases in height to a considerable degree in response to stimulation by anterior pituitary substance. It usually becomes high columnar, and quantitative determinations show that the epithelial cell undergoes marked hypertrophy (table 1, p. 119). Hyperplasia is likewise indicated, and is apparently more readily achievable in the female than in the male. In two cases of administration of anterior pituitary substance (both females) the hyperplastic condition induced in the thyroid gland was evidenced by the presence, in the latter, of numerous mitoses.

The intrafollicular colloid, in response to anterior pituitary treatment, assumes a foamy, highly vacuolated appearance, and eventually disappears altogether from the follicular lumen (fig. 7). The cytoplasm of the epithelial cell becomes finely granular, and relatively large colloid spherules are sometimes encountered in it.

The method found to be most effective for thyroid stimulation by anterior pituitary substance was the injection of pituitary hebin; the Parke, Davis sheep pituitary suspension was also thyreotropically potent. Next in thyroid stimulating power was the implantation of rat anterior pituitaries where-

as a similar treatment with guinea pig pituitaries, even when the latter were removed from castrated animals, proved to be entirely ineffective.

While injection of pituitary hebin and of the suspension of sheep pituitary powder resulted, in both male and female ground squirrels, in a considerable increase in height of the follicular epithelium and in elimination of the intrafollicular colloid, prolonged administration of the same preparations, at least in two males and three females, tended to revert the epithelium to a lower, apparently actively storing type, and to refill the follicles with a thin, pale staining, bubbly secretion resembling the colloid normally observed in the thyroids of animals sacrificed during the April to October period (fig. 8).

Associated with the histological changes produced in the thyroid under the influence of artificial pituitary stimulation is, particularly in females, an enormous increase of thyroid weight. In individual cases, this increase in thyroid weight amounted to as much as 400 per cent. It was greatest in animals injected with the liquid pituitary preparations; it was significant, though not as striking, in animals which had received implants of rat anterior pituitaries, and was completely lacking in recipients of guinea pig hypophyses. Excluding the latter category, the mean thyroid weight in eighteen treated females was 20.1 mg. whereas the corresponding average for an equal number of comparable untreated females amounted to 9.6 mg. The average increase in thyroid weight in the experimental females was well over 200 per cent. The number of males treated with anterior pituitary substances was insufficient for similar quantitative determinations; also here, however, thyroid enlargement was considerable.

All other gonadal stimulants used (untreated urine of pregnant women, urinary hebin and antuitrin-S) had no observable effect on the thyroid gland.

b. Castration and administration of sex hormone preparations

The lot of total castrates included in this investigation consisted of thirty-seven males and seventeen females. The duration of the state of castration in the males ranged from 4 days to about 7 months, and in the females from 1 to 3 $\frac{3}{4}$ months. The castrates were sacrificed, singly or severally, at various periods of the annual cycle. Since the greatest majority of the castrates had been in laboratory confinement prior to being sacrificed, for periods of time exceeding 1 month, any inhibition of thyroid activity that might have been produced by castration would be undetectable as even in untreated laboratory confined animals the thyroid was on a low level of activity throughout the year. On the other hand, any thyroid stimulation which might be caused by castration would, for this very reason, be properly demonstrable. Such stimulation, however, failed, with two doubtful exceptions,⁷ to disclose itself. Histologically, the thyroid follicles of the castrates showed the same low, sluggish epithelium and dense, deep staining intrafollicular colloid as the acini of untreated laboratory confined animals. The post-operative gain in body weight and deposition of subcutaneous and intraperitoneal fat were not greater in castrates than in normal ground squirrels kept for equal periods of time in captivity.

It is, however, the feeling of the writer that the matter of the influence of castration on thyroid activity needs further investigation on laboratory animals better suited for this purpose than the ground squirrel where the effect of laboratory confinement enters as a disturbing factor.

In either sex, injection of testis hormone or oestrin had no detectable effect on the thyroid gland.

⁷ Which might have represented pathological conditions not altogether uncommon among laboratory confined ground squirrels.

DISCUSSION

The present investigation, involving some 350 males and a similar number of females of the ground squirrel, *Citellus tridecemlineatus*, has demonstrated the presence of two major phases in the annual cycle of its thyroid gland, namely: a phase of relatively low activity during the hibernation period, and a phase of relatively high activity during the non-hibernating season. The seasonal variations in the activity of the thyroid gland in *Citellus tridecemlineatus* seem, therefore, to be concerned primarily with the regulation of the animal's general activity and metabolism and would undoubtedly obscure any interrelationship which might exist secondarily between the thyroid gland and sexual periodicity.

Needless to say, we are fully ignorant of the nature of the hormonal or perhaps neuro-hormonal complex underlying the annual thyroid cycle in a wild, hibernating vertebrate. That the anterior lobe of the hypophysis constitutes a factor of some significance in this complex, is evident from the fact that, at least in *Citellus tridecemlineatus*, the writer was able to reproduce in the winter thyroid, histological pictures practically identical with activity phases displayed by the animal's thyroid during the non-hibernating period, by administration of anterior pituitary substance.

Recent investigations, moreover, point to a reciprocal relationship between the anterior pituitary and thyroid gland not unlike the interaction postulated by Moore and Price ('32) for the anterior lobe of the hypophysis and the gonads. Thus, Houssay, Biassoti and Magdalena ('31) have shown that regressive changes occur in the thyroids of hypophysectomized dogs. Similar results were obtained by Smith ('30) in the rat. Conversely, Hohlweg and Junkman ('33) and Severinghaus, Smelser and Clark ('34b) have described, in the rat, conspicuous histological changes in the anterior pituitary following total thyroidectomy. According to Hohlweg and Junkman, administration of thyroid substance or thyroxin to thyroidectomized animals definitely prevents the appearance of these hypophyseal changes. Specific histological and cyto-

logical changes in the anterior pituitary of the adult male rat were also observed by Severinghaus, Smelser and Clark ('34 a) following thyroid feeding or administration of thyroxin.

The gonadal cycle does not seem to constitute a factor significantly involved in thyroid periodicity. Laboratory confined males that come into breeding condition during the latter half of the winter do not show, histologically, a thyroid picture essentially different from that of males with abdominal testes and minimal or near minimal accessories; on the other hand, both males and females continue on a high level of thyroid activity during the latter half of the summer and during the early autumn, although their reproductive organs, at that time, are regressed to the fullest extent. Furthermore, modification of the reproductive cycle by administration of sex hormones or gonad stimulating preparations derived from human pregnancy urine, or by castration, produced no detectable effect on the thyroid gland. Whether, conversely, experimentally produced disturbances in thyroid activity will affect the sexual cycle of our ground squirrel, is a problem now occupying the attention of the writer, and the results of this phase of his investigation will shortly appear in publication.

It should be noted, in connection with the present discussion, that both Stender ('24), in his investigation of the seasonal variations in the thyroid of the pig, and Geuer ('31), in his study of the annual thyroid cycle in the horse, likewise report a lack of significant correlation between the sexual rhythm and thyroid periodicity. Sklower ('25), in the frog, associates thyroid activity primarily with the seasonal fluctuations in general metabolism and attributes to the thyroid gland great physiological significance in the onset and maintenance of the hibernating state.

The hypertrophy and hyperplasia of the thyroid gland in pregnancy, a fact now established on a wide variety of mammalian species and verified in this investigation on *Citellus tridecemlineatus*, still awaits a satisfactory explanation. The

factors responsible for these pregnancy changes in the thyroid are absent in human pregnancy urine, as neither raw urine of pregnancy nor extracts prepared from it have any appreciable effect on the thyroid. These findings are in complete accord with the negative results obtained by Loeb ('31) in the thyroid of the immature guinea pig by injection of human pregnancy urine, and by Jannsen and Loeser ('32) in the young guinea pig and dog through administration of gonad stimulating extracts made from urine of pregnant women.

The fact, however, that the pregnancy changes in the *Citellus* thyroid continue, though to a less spectacular degree and in a progressively decreasing intensity, also during lactation, would seem to link the thyroid hyperactivity in question with the course of development and decline of the corpora lutea of pregnancy. Furthermore, thyroids of females with follicle ripening ovaries (proestrus, oestrus) show neither hypertrophy nor hyperplasia whereas thyroids of females with strongly luteinized ovaries (mid and late pregnancy, early lactation) markedly exhibit both. These observations lend themselves ideally to a correlation with the experimental findings of Greep ('33) that it is the luteinizing, and not the follicle ripening, fraction of the anterior pituitary which activates the thyroid gland. We are thus in possession of several suggestive facts pointing in the same direction; further experimental evidence will soon definitely settle the point.

SUMMARY AND CONCLUSIONS

A study of the seasonal changes in the thyroid gland of the ground squirrel, *Citellus tridecemlineatus*, fails to reveal any significant interrelationship between the animal's reproductive cycle and the thyroid gland. The latter follows its rhythm of lower activity during the hibernation period (November to March) and higher activity during the non-hibernating season (April to October). The seasonal variations in the thyroid gland seem, therefore, to be concerned primarily with the regulation of the animal's general activity and metabolism, and would undoubtedly obscure any interaction which might

exist secondarily between the thyroid gland and sexual periodicity. By administration of anterior pituitary substance during the period of low thyroid activity, the latter can be stimulated to reach and even surpass the April to October level. It appears, then, that the anterior lobe of the hypophysis constitutes a factor of significance in the manifestation of the thyroid rhythm.

In the female, the period of highest thyroid activity, accompanied by hypertrophy of the gland and severe hyperplasia within it, coincides with pregnancy and, to a somewhat lesser degree, also with lactation.

Castration, administration of sex hormones, of human pregnancy urine, or of gonad stimulating preparations derived from human pregnancy urine, had no observable effect on the thyroid.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

3 A typical 'resting' winter thyroid (December). Note the low epithelium and the dense colloid. Between the two follicles at the top of the figure is an interfollicular cell islet. Formalin-Zenker; Krichesky-Mallory. $\times 355$.

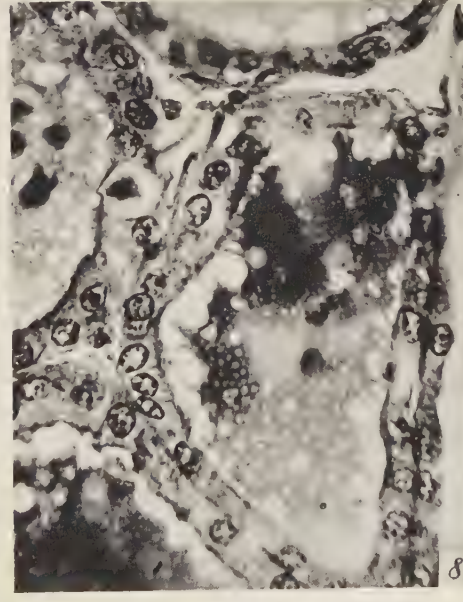
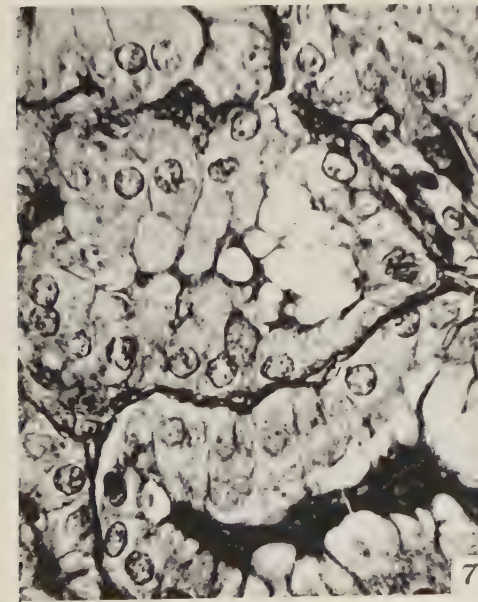
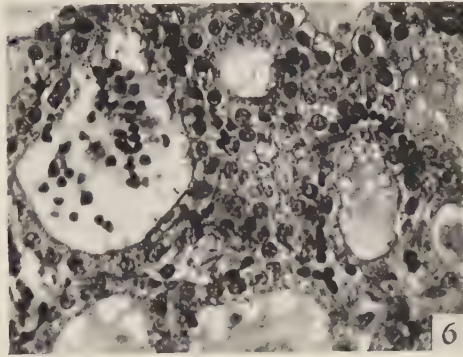
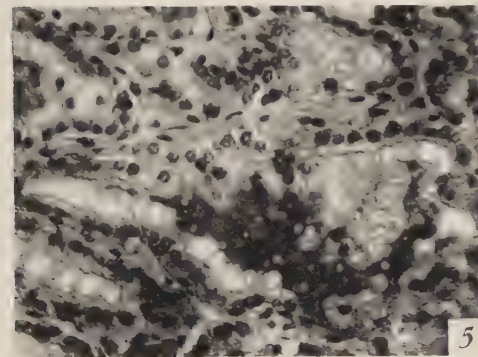
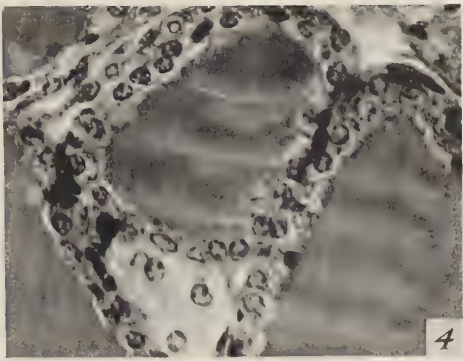
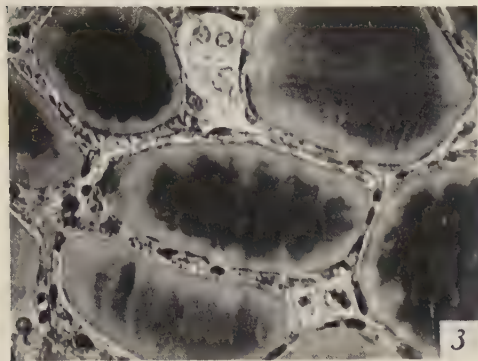
4 A 'storing' winter thyroid (December). Note the cuboidal epithelium and the dense colloid. Apical colloid globules may be discerned in some epithelial cells. An interfollicular islet appears near the center of the lower margin of the field. Formalin-Zenker; Krichesky-Mallory. $\times 475$.

5 Thyroid of a female in late pregnancy (May). Note the extreme hyperplasia in the gland. Bouin; Krichesky-Mallory. $\times 275$.

6 Thyroid of an actively lactating female (June). Note the high degree of hyperplasia and colloid exhaustion. Formalin-Zenker; Krichesky-Mallory. $\times 355$.

7 Photomicrograph of a portion of a thyroid at the height of colloid exhaustion. Male no. 53; thirteen daily injections of pituitary hebin, $1\frac{1}{2}$ cc. per day. Note the high columnar epithelium, collapsed follicles, and 'foamy' colloid. Bouin; Krichesky-Mallory. $\times 620$.

8 Photomicrograph of a portion of a thyroid re-secreting thin, bubbly colloid into the follicular lumina. Female no. 298; eight daily injections of sheep pituitary suspension, 1 cc. per day. Note the lowered epithelium as compared with that shown in figure 7. Epithelial cell in mitosis in upper left. Bouin; Krichesky-Mallory. $\times 620$.



A STUDY OF THE SEASONAL CHANGES IN THE
ADRENAL GLAND OF THE THIRTEEN-LINED
GROUND SQUIRREL (*CITELLUS TRIDECIMLINE-
ATUS*), WITH PARTICULAR REFERENCE TO ITS
SEXUAL CYCLE ¹

MOSES ZALESKY

Hull Zoölogical Laboratory, The University of Chicago

ONE CHART AND TWO PLATES (SEVEN FIGURES)

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INTRODUCTION

The literature dealing with endocrine interaction in general and with adrenal-gonad interrelationships in particular presents a bewildering confusion of disharmonious findings. Thus, a casual glance through the tabular summaries included in chapter XVII of 'Sex and Internal Secretions'² will suffice to impress one with the inconsistency of the results obtained thus far in the investigation of the effects of bi-

¹ This investigation has been aided by a grant from the Committee on Research in Problems of Sex of the National Research Council; grant administered by Prof. F. R. Lillie.

² This chapter is a contribution of Severinghaus, Engle and Smith to the volume named above, and is listed in 'Literature cited' under the authors' names.

lateral adrenalectomy upon the reproductive system. As for the effect of administration of adreno-cortical substance on the gonads, the corresponding reports are likewise extremely contradictory. Eaton et al ('29) find an increase in the weight of testes of young chicks fed with desiccated adrenal cortex. Chidester, Eaton and Thompson ('29) conclude that young female rats fed 0.5 gm. of desiccated adrenal cortex daily reach sexual maturity sooner than untreated controls or litter-mate females fed 0.5 gm. desiccated adrenal medulla daily. Engelhart ('30), using a lipoid cortical extract, obtained in young female rabbits a marked hypertrophy of the uterine mucosa and musculature. Müller ('31), however, injected immature female rats with a protein-free extract of adrenal cortex of beef and found that the experimental animals came into puberty considerably later than the controls and that the entire reproductive tract of the former was markedly atrophied. The same extract used by Klein ('31) on young male rats stimulated greatly their testes and reproductive accessories and induced precocious sexual development. Corey and Britton ('31) reported precocious maturation of the gonads of young albino rats following injection of a cortico-adrenal extract prepared by a modified Swingle-Piffner method. According to Corey and Britton, females responded to the treatment more effectively than males. Gaunt and Parkins ('33), however, working on rats and chicks, were unable to confirm these observations. Atwell ('32) injected hypophysectomized tadpoles of *Rana sylvatica* with Hartman's cortin, with the result that the ovaries of the treated animals averaged one and one-half and two times the size of ovaries from untreated hypophysectomized controls. This result suggests that the adrenal cortical hormone exercises a stimulating action on the ovary directly or, at least, not through the intermediary of the hypophysis.

In view of these and many other conflicting and indefinite results, it was decided in this laboratory to attack the problem of the adrenal-gonad interrelationship from an entirely different, obviously promising, though hitherto overlooked angle.

The assumption underlying our decision was that any significant interaction which might exist between the adrenal gland and the gonads ought to be demonstrable seasonally in an annually breeding wild mammal. It was, furthermore, realized that this type of test for the adrenal-gonad interrelationship would evidently be more critical, more fundamental, and more conclusive than experimental procedures involving administration of extracts of unknown chemical composition and, therefore, of doubtful purity, or removal of a gland so essential to life that its extirpation profoundly and complicatingly upsets the entire organism.

Our choice for this seasonal study fell upon the thirteen-lined ground squirrel (*Citellus tridecemlineatus*), an annually breeding mammal which offers the additional advantages of convenient size, fairly good tolerance of captivity, and relative abundance in the Chicago region.

It was anticipated that the seasonal changes in the endocrine glands of this animal would furnish the desired basic approach to the problem of interglandular relationships. Accordingly, the seasonal variations in the adrenal gland of the thirteen-lined ground squirrel constitute the chief object of this study; in addition, however, an experimental analysis of some of the relationships involved was attempted, and its results were likewise included in this report.

The present investigation constitutes a part of a co-operative study of the physiology of reproduction in relation to the endocrine glands. A summary of the results of this general project is presented elsewhere (Moore, Simmons, Wells, Zalesky and Nelson, '34). The project was initiated and directed by Prof. Carl R. Moore, for whose kind and instructive guidance throughout the course of this work the writer is profoundly grateful. The reproductive cycle and its experimental modification in the female were studied by Mr. Geo. F. Simmons; those in the male, by Mr. L. J. Wells, and the hypophysis of both sexes with regard to the sexual phenomena under investigation, by Dr. W. O. Nelson. Dur-

ing the experimental phases of this study a great many hormone preparations have been utilized. It is, then, with great pleasure that the writer makes the following acknowledgments: to Dr. H. B. Van Dyke and Mrs. Z. Wallen-Lawrence, of the Department of Physiological Chemistry and Pharmacology, The University of Chicago, for pituitary hebin extracted from dried sheep pituitary powder, and to Dr. T. F. Gallagher and Prof. F. C. Koch, of the same department, for testis hormone (bull testis extract); to Dr. R. G. Gustavson, of the University of Denver, for oestrin (placental and pregnancy urine extracts); and to Dr. Oliver Kamm, of Parke, Davis & Co., for antuitrin S and whole sheep pituitary suspension, kindly furnished through the courtesy of Dr. W. O. Nelson. Untreated human pregnancy urine, collected locally, was likewise used.

The writer's sincere thanks are also due to Mr. Geo. F. Simmons and Mr. L. J. Wells for their splendid, hearty cooperation and invaluable general assistance; and to Dr. Normand Hoerr and Dr. W. O. Nelson, for their helpful suggestions regarding various technical procedures employed in this study.

MATERIAL AND METHODS

The glands treated in this investigation were removed at autopsy from animals used by Mr. Simmons and Mr. Wells in their study of the sexual aspect of the general project. Since their reports, to be published in the near future, include a detailed description of the conditions under which the laboratory confined animals were kept, these particulars have been omitted from the present paper. Most of the seasonal work, however, was done on animals killed shortly following their capture.

The weighing of the glands was accomplished by means of an analytical balance. The tissue—one adrenal from each animal sacrificed³—was weighed after fixation in Bouin's fluid while submerged in the latter.

³ The bilateral differences in the weight of the glands were generally found to be rather insignificant.

The fixing agents employed were chiefly Bouin's fluid and formalin-Zenker, the latter having been prepared according to the formula used by Hoerr ('31). Whereas Bouin fixation gave dependable results for general histological structure and relationships, the finer cytological details were brought out more satisfactorily in the formalin-Zenker-fixed material. Paraffin sections were obtained from 3 to 5 μ in thickness. The staining was done chiefly with Krichesky's ('31) modification of the Mallory connective tissue stain, which proved excellent for the adrenal; hematoxylin (Harris' modification) with eosin counterstaining was also used.

The method found to be most effective and reliable for the preservation and staining of the adrenal lipoids involved fixation in Regaud's fluid and staining in Romeis' ('29) sudan orange. This valuable method was suggested to the writer by Dr. N. Hoerr and is described fully in one of the latter's forthcoming publications.

The Reese ('31) modification of the Bell ('29) osmic method for lipoids also gave good, though less dependable results.

GENERAL HISTOLOGY OF THE GLAND

The adrenal gland of the thirteen-lined ground squirrel presents several peculiarities, and, therefore, a more or less detailed description of its general histology is deemed desirable.

The outer investment of the gland consists of a fibrous capsule of varying thickness. The zona glomerulosa is usually narrow, and has never been observed to exceed one-sixth of the total width of the adrenal cortex. Its cells are normally closely packed within the spheroidal glomeruli, with apparently little interglomerular connective tissue. The fibrous radial trabeculae dividing the glomeruli are, however, conspicuous, and often extend into the zona fasciculata.

The typical glomerulosa cell has a small, spherical, centrally placed and densely chromatined nucleus; a limited amount of cytoplasm which, in glands prepared by ordinary histological methods, is more or less vacuolated; and usually

indistinct cell walls. The cytoplasmic vacuolization, from comparisons made with Romeis lipid preserving preparations, appears to be due to the dissolution of lipoids by the acetic acid fixatives, alcohols, and high temperature paraffine infiltration employed in the histological routine. Usually the inner half of the glomerulosa is more intensely vacuolated.

The zona fasciculata of the adrenal cortex is highly variable in thickness and is often transitional in character, gradually blending the zona glomerulosa into the reticularis and making the zone limits within the cortex extremely indistinct. Whereas in other mammals (e.g., man, dog) the fasciculata is the widest, best developed zone, in the thirteen-lined ground squirrel it is usually narrow and inconspicuous, and, under certain conditions specified below, it cannot be definitely identified. Whenever present and clear-cut, the fasciculate zone answers the description of the 'spongiosa' of authors. It consists of large cells attaining up to 35μ in diameter, spherical or polyhedral in shape, with a considerable amount of flocculently granular, more or less intensely vacuolated cytoplasm, and a large, round, vesicular nucleus containing a loose chromatin network and usually showing one to two nucleoli. Also here the vacuolization is traceable to lipoids dissolved by ordinary histological technique. The fasciculata cells are arranged in a fashion typical of this zone in all mammalian adrenals, namely, in cords radially disposed and separated from each other by connective tissue and narrow sinusoids.

The most outstanding layer of the adrenal cortex, in the animal under consideration, is the reticular. In adult animals it usually represents the widest cortical zone, highly conspicuous by the abundance of dense, deeply staining material (pigment?) in the cytoplasm.

The typical reticularis cells are smaller than the fasciculata cells, are spheroidal or cuboidal in shape, and their nuclei resemble those of fasciculata cells. The arrangement of cells in the typical reticularis is also identical with that observed in the corresponding layer of other mammalian adrenals: the

cell cords here anastomose in an irregular fashion and are again divided by stroma tissue and blood sinuses.

The adrenal medulla is variously shaped and variously located within the gland—a situation anticipated from the embryology of the adrenal chromaffine system. Accessory medullary nodules are often found embedded in the cortex. The main medullary mass may be eccentric and even reach the periphery of the gland, particularly at the hilus. Such medullary outgrowths into the cortex usually disturb the zonation in the adjacent cortical areas. The differentiation of reticularis cells into the so-called 'dark' and 'light' cells described, for the guinea pig, in detail by Hoerr ('31) and occasionally encountered in the adrenal of *Citellus tridecemlineatus*, is invariably more pronounced in the vicinity of the medullary 'peninsulae.'

The medulla consists of variously shaped and irregularly arranged cell masses separated by fibrous connective tissue and by large sinuses in which one finds abundant erythrocytes, debris of destroyed cortical cells, and a substance staining a homogeneous pale blue with Mallory, probably a coagulum of blood plasma. A medullary cell is about the size of a fasciculata cell, elongated, more or less distinctly cuboidal or somewhat triangular in shape, with a vesicular nucleus showing one to four nucleoli. The nucleus is usually centrally located within the cell; it may, however, be shifted toward either pole of the long cellular axis. The immediate proximity of a blood sinus does not seem to influence the position of the nucleus within the cell. The cytoplasm of the medullary cell contains extremely fine granules staining light purple with Mallory. Whether this granulation is of any significance in the elaboration of the chromophilic material is unknown. Islets composed of cortical cells, most closely resembling those of the zona reticularis, are also found here and there among the cords of medullary cells. Occasionally one is fortunate enough to obtain a section through the medulla at a level containing an unusually large sympathetic ganglion which may show as many as some twenty relatively huge, irregularly shaped cells with a deeply stain-

ing cytoplasm and a good-sized nucleus. Sympathetic ganglia may rarely be extra-medullary and even extra-capsular in location.

Small accessory adrenals consisting exclusively of cortical tissue are not infrequently found in the vicinity of the main gland. Whether accessory adrenals may also be located in other regions of the abdominal cavity, was not ascertained.

The distribution of lipoids in the normal adrenal is as follows: there is usually a very narrow peripheral layer poor in lipoids, or entirely devoid of them. The cytoplasm of the inner glomerulosa and particularly of the entire fasciculata is packed with varying large spherical droplets of lipid material which take sudan orange most intensely. The lipid content of the reticular zone and of the cortical islets of the medulla is low and probably finely divided and homogeneously distributed throughout the cytoplasm. The medulla proper does not take the stain at all and is thus devoid of lipoids and fats which have an affinity for sudan orange. Occasionally large spherules of lipid material are seen scattered throughout the inner reticularis, in close proximity to the medulla. They are probably associated with focal degeneration which is most pronounced at this level.

A systematic destruction of presumably senescent cortical cells is carried on in the inner reticularis, in close proximity to the medulla. This innermost layer of the adrenal cortex often shows a ring of connective tissue fibers entangling cells in various stages of degeneration; hyperchromatic, pycnotic, and disintegrating nuclei; and debris of all sorts. The adjacent medullary sinuses likewise represent a veritable 'dumping ground' for cellular remains apparently derived from the cortex and eventually removed by the blood stream.

Mitotic figures are exceedingly rare in histological preparations of *Citellus* adrenals. In spite of a most thorough search, the writer found only two instances of mitotic division among some 600 adrenals examined. The two mitoses observed were in the glomerular zone. Thus, regeneration in the adrenal gland could not be directly observed. The work of other students of the mammalian adrenal, notably of

Graham ('16) and Hoerr ('31) offers, however, satisfactory evidence to the effect that cells proliferate in the peripheral layers of the adrenal cortex and migrate inward to replenish the losses sustained at the medullary border.

SEASONAL VARIATIONS IN THE ADRENAL OF CITELLUS
TRIDECEMPLINEATUS

The following quotation from the report on the general project of which this study is a part (Moore, Simmons, Wells, Zalesky and Nelson, '34) will familiarize the reader with the essential features of the sexual cycle in *Citellus tridecemlineatus*:

. . . . Female ground squirrels emerge from hibernation during the early days of spring, usually about the third week in April, some ten days later than the males. A single period of oestrus occurs, lasting only until coitus a few days after emergence Gestation occupies four weeks, and litters are delivered from mid-May to the first week in June. The reproductive tract undergoes the greater part of its involution immediately after parturition, though during the four to six weeks of lactation the ovaries actually increase in size; after weaning, the ovaries and all accessories gradually regress to a low point, and remain essentially inactive during summer and fall. About January 1, while females are in mid hibernation, the ovaries begin to show signs of activity, followed by a slow gradual development of oviducts, uterus, vagina, and mammary glands toward their peak in spring. . . .

. . . . Males usually appear above ground during the first 10 days of April, when some are in mating condition. Testes are large and scrotal, and contain spermatozoa, while reproductive accessories are approaching the peak of functional maximum reached in most males by late April. Usually by July 1 the testes have declined in size and activity and have receded into the abdominal cavity, and spermatozoa have disappeared from the epididymis; all accessories, including epididymis, vas deferens, seminal vesicles, prostate, Cowper's and bulbar glands, etc., have undergone regression, and remain more or less quiescent during the first part of hibernation, which begins in October. Spermatogenesis gradually becomes evident in December, with spermatozoa appearing in seminiferous tubules in January or February. The testes are essentially aspermatic from July to January. . . .

The adrenal cortex of the thirteen-lined ground squirrel exhibits seasonal changes which can be definitely correlated with the animal's annual sexual cycle. All histological method employed in the course of the present investigation failed, however, to disclose any seasonal variations in the adrenal medulla.

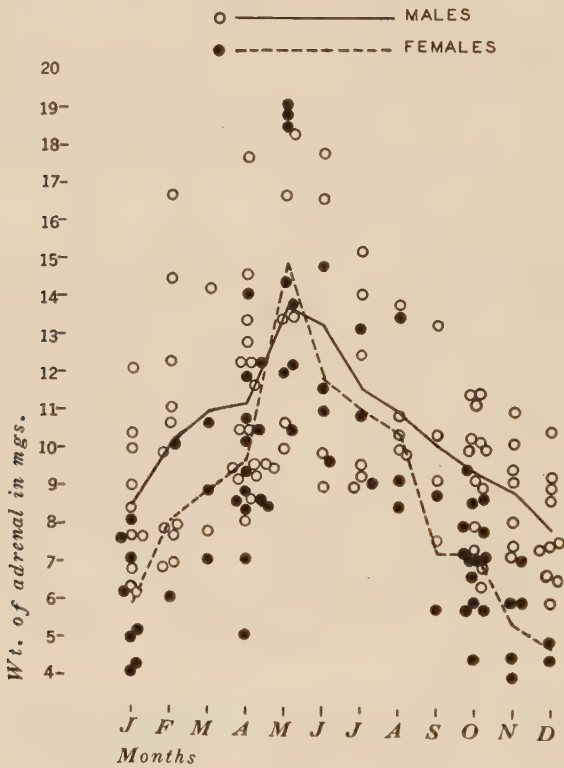


Chart 1 Seasonal fluctuations in the weight of the adrenal gland of *Citellus tridecemlineatus*.

Seasonal fluctuations in the weight of the adrenal gland show, in both sexes, a distinct seasonal trend. The curve, as plotted against the months of the year, gradually ascends from the low winter point to a May peak, and thence slowly returns to the winter bottom (chart 1).

Determinations of the cortex-to-medulla ratio were made in glands representative each of a different season of the

year. The following method was used: each gland selected for this purpose was sectioned serially and transversely at 10μ , and an outline drawing made on paper, by means of a camera lucida ('Zeichenapparat nach Abbé'), of every tenth section of that gland. The drawing, in each case, included the circumference of the entire section as well as of its medullary portion whenever present. The total area of the magnified sections drawn for a given gland was then determined by means of a Keuffel-Esser planimeter, and similar measurements were taken of the corresponding medullary area. The number of planimeter units of area contained in the magnified medullary portion as it appeared in the drawings (designated in table 1 as 'M') was subtracted from the number of similar units obtained for the entire gland, and the remainder gave the number of planimeter units of cortical area (symbolized by 'C' in table 1) in the outline drawings of the adrenal under consideration. The ratio of the numbers of planimeter units obtained for these enlarged areas of cortex and medulla, respectively, being also the ratio of the true volume of cortex to the true volume of medulla, was simplified by reducing the latter to unity (these ratios, in their simplified form, appear in table 1 in the column headed 'C/M'). These determinations must not, of course, be regarded statistically: they merely offer suggestive comparisons. Thus, they indicate that, in both sexes, the increase in the weight of the gland in the spring is due chiefly to enlargement of cortical volume. It is noteworthy, moreover, that this increase in the volume of the cortex is greatest during the period of the highest sexual activity and, in the female, also during gestation (table 1).

The data given in table 1 reveal an interesting relationship. On the male side, for instance, the cortex-medulla ratio in the winter animal with abdominal testes and undeveloped accessories⁴ (no. 741) was 5.9:1 while in the spring animal

⁴Throughout the present report, all references to the sexual condition of the males and females studied are based on diagnoses made by Mr. L. J. Wells and Mr. Geo. F. Simmons, respectively.

TABLE 1

Cortex-medulla ratios in the adrenal at various periods of the annual sexual cycle

ANIMAL (M-MALE, F-FEMALE)	DATE SACRI- FICED	C	M	C/M	REMARKS
M-741	12/ 3/33	12,042	2067	5.9:1	Hibernating, sexually low
M-749	1/ 6/34	10,603	1866	5.7:1	Laboratory adult, sexually low
M-627	4/30/32	21,243	3110	6.8:1	Testes and accessories still high, beginning to regress
M-750	1/ 6/34	37,934	3415	11.1:1	Laboratory adult, near breeding condition
F-268	11/21/32	11,699	2263	5.2:1	Sexually low
F-121	4/29/32	20,532	3120	6.6:1	In full oestrus
F-600	5/31/33	21,033	1923	10.9:1	Pregnant, near term

no. 627, just past breeding condition, the corresponding ratio amounted to 6.8:1. Particularly instructive was the case of males no. 749 and no. 750. Both were laboratory confined adult males, i.e., males which had passed at least once through a complete annual sexual cycle; both were sacrificed on the same day, namely, on January 6, 1934. Whereas, however, the former had small abdominal testes and low accessories, the testicles of the latter were of near maximum or maximum size and confined within the densely pigmented scrotum, the epididymis contained quantities of motile spermatozoa, and the remaining accessories were well advanced in their upward development—a contrast commonly observed among laboratory confined adult males during January and invariably paralleled by a corresponding difference in the size of the adrenals. Thus, the adrenal of male no. 749 weighed only 9 mg. and gave a cortex-medulla ratio of 5.7:1, while the respective gland of male no. 750 weighed 22.5 mg., and its cortex-medulla ratio was 11.1:1.

On the female side, the ratio of cortex to medulla in the sexually low November animal no. 268 was 5.2:1; in the April animal, which was in full oestrus, it rose to 6.6:1; and in female no. 600, in late pregnancy, it jumped further to 10.9:1.

A critical investigation into the immediate causes of the increase in cortical volume during the period of high sexual development was not undertaken as it would involve a prohibitive amount of cell counting. In the spring, with the onset of breeding activity, certain marked histological changes occur, however, in the adrenal cortex, which can account, at least partly, for the increase in volume of the latter. The cortex, as a whole, becomes highly hyperaemic. The zona reticularis widens and becomes differentiated into two distinct sub-zones which, until the physiological significance of this differentiation is known, we shall designate by the non-committal terms of 'reticularis A' and 'reticularis B.' The latter corresponds to the inner sub-zone which, in arrangement and cell structure, constitutes the typical reticularis described above and present in the gland throughout the year. The outer sub-zone, the 'reticularis A,' is, as we shall see later, transitory and attains its highest development in both sexes during the peak of sexual development. It forms chiefly, and perhaps wholly, at the expense of the fasciculata and still retains the fasciculate arrangement of its cell cords. Nevertheless, cytologically, each individual cell of this sub-zone is a reticularis cell, its reticularis characters, though, being exaggerated, and the entire cell apparently reaching a high degree of specialization.

A well-developed 'reticularis A' cell (fig. 5) is very large, often measuring $60\ \mu$ in diameter. It is polyhedral, cuboidal, or roughly triangular in shape. Its nucleus is similar to that of a typical fasciculata cell, the general increase in the size of the cell being due to cytoplasmic expansion. Surrounding the nucleus and often separated from it by a narrow protoplasmic ring, is a large mass of exceedingly dense, deep staining pigment-like material often bearing upon it a crescentic adnuclear cap reticular in structure. This dense pigment-like mass fills nearly the entire cytoplasm, leaving, however, a clear peripheral ring whose nature and physiological significance were not disclosed. When first noted in sections prepared from Bouin-fixed material, this peripheral ring was

thought to be a fixation artifact. The same structure appeared, however, in glands fixed in formalin-Zenker or Regaud's fluid as well as in lipoid-demonstrating preparations. Consequently, the peripheral ring is not a fixation product or a result of lipoid removal. Aside from the tendency of Bouin fixation to emphasize the contrast in staining properties between the light perinuclear and peripheral rings, on one hand, and the dark pigment-like mass, on the other, there was a surprisingly close correspondence in the cytological appearance of the 'reticularis A' cell regardless of the fixing and staining methods employed. Moreover, the essential constituents of the cell in question were identifiable even in unstained sections. The inevitable conclusion, then, is that the configuration of the substances of various densities in the 'reticularis A' cell is due to pre-existing chemico-physical differences, and not to artificially produced local disturbances of the lipoid-protein equilibrium. In its low lipoid content, the 'reticularis A' cell resembles the typical reticularis ('reticularis B') cell.

The width of the 'reticularis A' sub-zone, in spring adrenals, varies from gland to gland. It usually ranges between three and fifteen cells in thickness and is inversely proportional to the width and degree of general development of the fasciculata. Whereas the adrenal gland of the female never seems to exhaust its fasciculate zone—which, incidentally, is better developed throughout the year than in the adrenal of the male—a high degree of development of 'reticularis A' in the adrenal of the male may be found in association with the virtual suppression of the typical fasciculate zone. In cases where both these zones are present, the transition between the zone containing typical fasciculata cells and that consisting of 'reticularis A' cells is gradual and expresses itself in a progressive loss of lipoids and acquisition of the pigment-like dark material characteristic of reticularis cells. Whether 'reticularis B' also contributes to the formation of 'reticularis A,' remains thus far unknown, though unlikely. The process—

Fasciculata $\rightarrow$ 'Reticularis A' $\rightarrow$ 'Reticularis B'

seems to be irreversible.

'Reticularis A' shows a most remarkable seasonal behavior. In the male, it reaches its highest development shortly following emergence from hibernation, and continues on a high level during May. It persists, but is decidedly on the decline during the summer, and normally disappears altogether about October. From October to January it is either absent or poorly developed in the adrenals of both immature and adult males. In January, its upgrade progress begins, and, following a gradual increase in width and accentuation of its specific characteristics, it returns to a new peak early in April, and the annual cycle is then repeated. This rhythm is even more pronounced in the female. During the late autumn and winter, females, both immature and adult, show no trace of 'reticularis A' in their adrenals (figs. 1 and 3). Females captured in the early spring, upon their emergence from the winter burrow, had adrenals with a well-developed outer reticularis ('reticularis A'), but this sub-zone was observed to attain its highest degree of development in May, i.e., during pregnancy. The 'reticularis A' of pregnancy is undoubtedly more conspicuous, both qualitatively and quantitatively, than at any other period of the annual cycle in untreated animals of either male or female sex (figs. 2 and 4). Following parturition, this transitory sub-zone undergoes a gradual regression until, in October, it becomes no longer distinguishable in cell characters from 'reticularis B.'

EXPERIMENTAL

a. Administration of gonad stimulating substances

All gonad stimulating substances used proved, on the whole, excellent stimulants for the adrenal cortex. In order of stimulating power with respect to the adrenal cortex, they were: pituitary hebin and suspension of whole sheep pituitary powder (of these unstandardized materials, probably large overdoses were administered), implants of anterior pituitary

lobes of rats, untreated human pregnancy urine (chiefly of late pregnancy), and antuitrin S. None of these substances seemed to affect histologically the adrenal medulla, at least not in a manner demonstrable by ordinary technique. No stimulation, either in the cortex or in the medulla, was obtained from implantation of guinea pig pituitaries, even when long time castrates were used as donors.

A significant increase in the weight of the adrenal takes place following the administration of the pituitary gonadal stimulants. In a series of eighteen experimental females, the adrenal gland from a treated animal weighed, on the average, 20.3 mg. against 9.7 mg. which constituted the mean adrenal weight in an equal number of comparable untreated animals. Although the number of males treated with anterior pituitary was insufficient for a satisfactory statistical study, yet also here indications point to an enormous increase in the weight of the adrenal gland as a result of the treatment.

A similar, statistically significant increase in adrenal weight followed the administration of raw human pregnancy urine and antuitrin S (tables 2 and 3, respectively).

Quantitative determinations of cortex-medulla ratios show that whereas the adrenal medulla may or may not increase in volume as a result of administration of the gonadal stimulants used in this investigation, the cortex enlarges tremendously (table 4). This quantitative effect seems to be largely, if not wholly, due to direct stimulation of the adrenal by the gonad stimulating substances in question, or at least not through the gonads, since the same high degree of increase in cortical volume was obtained by injection of sheep pituitary suspension into several spayed females.

Histologically, the increase in cortical volume under the influence of the gonadal stimulants administered, can be traced chiefly to a considerable, most evident increase in the number of cells of the adrenal cortex, particularly striking in the reticularis; to an increased vascularization of the gland as a whole; to cellular hypertrophy in the glomerular and fasciculate zones; and to the differentiation of a wide, beautiful

'reticularis A,' irrespective of season. Hypertrophied glomerulosa cells elongate in a direction perpendicular to the adjacent trabecula, and their cytoplasm becomes intensely vacuolated due to an increase in lipoid content. Each cell of the fasciculate zone also expands in all directions, and its lipoid load is likewise increased. In the case of several males injected with pregnancy urine, however, the fasciculate zone was completely eliminated by the over-developed 'reticularis

TABLE 2
Effect of injection of untreated human pregnancy urine on adrenal weight

EXPERIMENTAL ANIMALS						UNTREATED CONTROLS			
Animal	Amount of daily injection	Injected for (days)	Sacrificed date	Body weight	Weight of adrenal	Animal	Sacrificed date	Body weight	Weight of adrenal
	cc.			gm.	mg.			gm.	mg.
♂ 35	2	40	12/12/31	167	10.0	♂ 43	12/12/31	196	7.4
♂ 36	2	33	12/ 5/31	118	14.4	♂ 744	12/ 3/33	226	7.6
♂ 322	2	22	8/26/32	148	14.2	♂ 330	8/22/32	180	16.8
♂ 323	2	30	9/ 3/32	120	34.7	♂ 721	9/ 9/33	234	8.6
♂ 325	1	15	8/24/32	96	17.9	♂ 717	8/18/33	154	12.3
♂ 326	1	31	9/10/32	122	22.2	♂ 727	9/ 9/33	150	7.5
♂ 331	2	10	9/15/32	114	11.4	♂ 728	9/ 9/33	152	8.2
♂ 332	2	13	9/18/32	116	21.1	♂ 715	8/18/33	144	10.2
♂ 334	2	12	9/18/32	122	25.3	♂ 716	8/18/33	134	10.1
♂ 335	2	22	9/28/33	154	13.4	♂ 386	10/30/32	142	6.9
♂ 336	2	47	10/29/32	150	20.8	♂ 387	10/30/32	168	9.2
♂ 354	2	41	11/19/32	144	15.6	♂ 734	11/19/33	202	11.0
♂ 359	2	41	11/19/32	192	17.3	♂ 735	11/19/33	230	9.5
♂ 360	2	41	11/19/32	148	9.1	♂ 736	11/19/33	228	9.2
♂ 362	2	41	11/19/32	156	11.3	♂ 424	10/17/32	206	9.0
♂ 364	2	38	11/19/32	196	8.4	♂ 737	11/19/33	216	10.2
♂ 396	2	27	12/10/32	152	13.9	♂ 435	12/28/32	195	6.7
♂ 397	2	27	12/10/32	173	16.4	♂ 742	12/ 3/32	272	11.5
Mean: 16.5 ¹						Mean: 9.6 ¹			
♀ 12	2	26½	11/ 3/31	144	16.7	♀ 45	11/21/31	187	4.0
♀ 29	3	19	11/18/31	146	8.3	♀ 46	11/22/31	166	6.0
♀ 49	3	10	1/16/32	172	22.8	♀ 269	11/21/32	185	7.1
♀ 220	2	3	8/31/32	120	17.4	♀ 665	8/18/33	122	9.5
♀ 267	2	60	11/21/32	130	13.3	♀ 270	11/22/31	202	4.5
Mean: 15.7 ²						Mean: 6.2 ²			

¹ Difference of means is 4.2 times its standard error; statistical significance of increase is less than 0.0001.

² Difference of means is 3.67 times its standard error; statistical significance of increase is 0.0002.

A,' and even the glomerulosa began to show a distinct tendency toward deglomerulization, acquisition of the deeply staining material typical of reticularis cells, and general transformation in the 'reticularis A' direction (fig. 6). In many greatly expanded 'reticularis A' cells of such adrenals the nuclear chromatin becomes clumped and shriveled, and is retracted from the nuclear membrane.

It should be noted that pregnancy urine had a generally toxic effect upon the animals, the greatest majority of which lost considerable weight and became highly emaciated as a result of this treatment. The injection sites turned into foci of spreading infection. Four males even succumbed to the treatment. It is doubtful, however, whether the toxicity of urine was significantly involved in the adrenal changes observed, since antuitrin S, a non-toxic extract made from urine of pregnant women, gave results practically identical with those obtained by injection of pregnancy urine.

TABLE 3
Effect of injection of antuitrin S on the weight of the adrenal gland

EXPERIMENTAL ANIMALS						UNTREATED CONTROLS			
Animal	Amount of daily injection	Injected for (days)	Sacrificed date	Body weight	Weight of adrenal	Animal	Sacrificed date	Body weight	Weight of adrenal
	<i>rat units</i>			<i>gm.</i>	<i>mg.</i>			<i>gm.</i>	<i>mg.</i>
♂ 350	50-100	29	11/ 6/32	146	15.4	♂ 393	11/12/32	182	7.4
♂ 358	50-100	21	10/28/32	143	16.0	♂ 386	10/30/32	142	6.9
♂ 394	100	27	12/10/32	214	11.1	♂ 43	12/12/31	196	7.4
♂ 395	100	27	12/10/32	236	11.4	♂ 435	12/28/32	195	6.7
♂ 428	15	26	1/ 4/33	203	10.2	♂ 458	1/28/32	250	10.4
♂ 429	35	26	1/ 4/33	171	5.7	♂ 749	1/ 6/34	150	9.0
♂ 430	5	26	1/ 4/33	160	13.4	♂ 56	1/16/32	154	7.7
Mean: 11.9 ¹						Mean: 7.9 ¹			
♀ 277	5	7	12/12/32	136	10.6	♀ 59	1/29/32	204	8.1
♀ 279	25	7	12/12/32	168	15.9	♀ 301	12/31/32	113	9.6
♀ 280	50	7	12/12/32	166	13.5	♀ 57	1/27/32	193	4.3
♀ 282	10	7	12/12/32	132	15.0	♀ 303	1/ 1/33	147	5.0
♀ 288	1	7	12/12/32	143	11.5	♀ 268	11/21/32	150	6.0
Mean: 13.3 ²						Mean: 6.6 ²			

¹ Difference of means is 2.84 times its standard error; statistical significance of increase is 0.0058.

² Difference of means is 4.75 times its standard error; statistical significance of increase is less than 0.0001.

b. Administration of sex hormones

The subcutaneous injection, into males or females, of either testicular or ovarian (follicular) hormone resulted in a slight, statistically insignificant increase in the mean weight of the adrenal gland (tables 4 and 5), and in variable cortex-medulla ratios (table 6).

In males injected with testis hormone there is, generally, no increase in the width of the adrenal cortex and there is no evidence of stimulated cell proliferation. A well-differentiated 'reticularis A' is developed, however, regardless of season. Furthermore, the degree of stimulation of 'reticu-

TABLE 4

Effect of injection of testis hormone on the weight of the adrenal gland

EXPERIMENTAL ANIMALS						UNTREATED CONTROLS			
Animal	Amount of daily injection	Injected for (days)	Sacrificed date	Body weight	Weight of adrenal	Animal	Sacrificed date	Body weight	Weight of adrenal
	<i>bird units</i>			<i>gm.</i>	<i>mg.</i>			<i>gm.</i>	<i>mg.</i>
♂ 34	12.5	25	12/27/31	180	9.6	♂ 734	11/19/33	202	11.0
♂ 103	6.6	9	7/19/32	144	25.8	♂ 707	7/17/33	100	5.6
♂ 105	13.3	20	8/ 1/32	204	16.3	♂ 718	8/18/33	236	13.9
♂ 106	13.3	20	8/ 1/32	194	9.4	♂ 330	8/21/32	180	16.8
♂ 107	6.6	7	7/20/32	170	14.9	♂ 708	7/17/33	210	15.2
♂ 113	7.0	22	8/16/32	106	7.0	♂ 714	8/18/33	132	11.0
♂ 115	6.6	21	8/11/32	228	23.8	♂ 718	8/18/33	236	11.9
♂ 116	6.6	21	8/11/32	220	10.1	♂ 719	8/18/33	212	10.9
♂ 351	12.0	12	10/20/32	116	10.0	♂ 386	10/30/32	142	6.9
♂ 352	6.0	29	11/ 6/32	170	6.5	♂ 735	11/19/33	230	9.5
♂ 388	12.0	26	11/26/32	222	16.9	♂ 737	11/19/33	216	10.2
♂ 489	15.0	21	4/23/32	173	19.4	♂ 619	4/21/33	194	12.8
♂ 490	15.0	26	4/29/32	165	12.0	♂ 625	4/29/33	159	12.3
Mean: 14.0 ¹						Mean: 11.4 ¹			
♀ 27	10.0	19	11/18/31	176	7.0	♀ 45	11/21/31	187	4.0
♀ 28	20.0	19	11/18/31	154	7.8	♀ 46	11/22/31	166	6.0
♀ 291 ²	5.0	7	12/12/32	143	7.0	♀ 301	12/31/32	113	9.6
♀ 292 ²	15.0	7	12/12/32	126	6.8	♀ 303	1/ 1/33	147	5.0
Mean: 7.2 ³						Mean: 6.2 ³			

¹ Difference of means is only 1.35 times its standard error; increase not statistically significant.

² Bilaterally spayed; injections begun immediately following ovariectomy.

³ Difference of means is less than its standard error; increase not statistically significant.

laris A' corresponds roughly to the amount of testis hormone administered. No noteworthy change in the lipid content of the glomerular and fasciculate zones was observed.

The adrenals of males which had received injections of oestrin showed, peculiarly enough, effects similar to those exhibited by the corresponding glands of males injected with testis hormone. In all males of this series, with the exception of one doubtful case, the adrenal presented a well-developed 'reticularis A.' The correlation between the amounts of hormone injected and the degree of stimulation of the 'reticularis A' was, however, not so apparent as in the case of the testis hormone treatment. Moreover, the highest level of 'reticu-

TABLE 5
Effect of injection of oestrin on the weight of the adrenal gland

EXPERIMENTAL ANIMALS						UNTREATED CONTROLS			
Animal	Amount of daily injection	Injected for (days)	Sacrificed date	Body weight	Weight of adrenal	Animal	Sacrificed date	Body weight	Weight of adrenal
	<i>rat units</i>			<i>gm.</i>	<i>mg.</i>			<i>gm.</i>	<i>mg.</i>
♂ 32	12.5	32	12/ 5/31	161	11.8	♂ 43	12/12/31	196	7.4
♂ 33	25.0	25	12/12/31	150	10.0	♂ 744	12/ 3/33	226	7.6
♂ 84	25.0	34	5/14/32	178	15.8	♂ 88	5/14/32	140	18.3
♂ 84	12.5	34	5/14/32	160	16.9	♂ 89	5/14/32	162	13.5
♂ 112	25.0	17	8/11/32	96	8.5	♂ 714	8/18/33	132	11.0
♂ 327	25.0	11	8/23/32	116	27.7	♂ 715	8/18/33	144	10.2
♂ 328	25.0	7	8/19/32	74	12.6	♂ 716	8/18/33	134	10.1
♂ 345	25.0	29	11/ 6/32	196	13.1	♂ 734	11/19/33	202	11.0
♂ 346	12.5	29	11/ 6/32	214	15.4	♂ 735	11/19/33	230	9.5
♂ 347	12.5	29	11/ 6/32	91	13.1	♂ 736	11/19/33	228	9.2
♂ 349	25.0	29	11/ 6/32	181	13.2	♂ 803	10/21/33	228	11.2
♂ 487	25.0	26	4/29/33	128	14.9	♂ 625	4/29/33	159	12.3
♂ 488	25.0	26	4/29/33	136	17.3	♂ 624	4/29/33	200	17.7
Mean: 14.6 ¹						Mean: 11.5 ¹			
♀ 25	12.5	19	11/18/31	144	7.6	♀ 45	11/21/31	187	4.0
♀ 26	25.0	19	11/18/31	178	10.7	♀ 46	11/21/31	166	6.0
♀ 293 ²	1.0	7	12/12/32	136	7.6	♀ 301	12/31/32	113	9.6
♀ 294 ²	6.0	7	12/12/32	144	7.5	♀ 303	1/ 1/33	147	5.0
Mean: 8.4 ³						Mean: 6.2 ³			

¹ Difference of means is only 1.94 times its standard error; increase not statistically significant.

² Bilaterally spayed; injections begun immediately following ovariectomy.

³ Difference of means is only 1.52 times its standard error; increase not statistically significant.

laris A' development was found, with one single exception, in animals captured and sacrificed during the spring and summer, i.e., at periods when a good outer reticular sub-zone is normally present in the adrenal. Obviously, the administration of oestrin in these cases may not have had any effect at all. It seems, therefore, that a more extensive series of oestrin injected males is necessary in order to establish the effect of follicular hormone on the adrenal cortex of the male *Citellus*.

One interesting fact, however, was revealed by the study of the adrenal in males injected with oestrin: the treatment caused injury, in some cases severe, to the cortical tissue. This injury was evidenced by the occurrence of large hemorrhagic areas in the peripheral zones, and by the prevalence of large intracellular vacuoles, of 'signet ring' cells and 'giant cells,' and of more or less extensive fibroses in the reticularis. The lipid content of the glomerulosa and of the fasciculata was markedly increased.

Among the females, only four were injected with oestrin, and four with testis hormone. Four of these females (two injected with testis hormone and two with oestrin) were sacrificed in November, and the remaining four in December. Whereas in controls of this time of the year 'reticularis A' is invariably absent from the adrenal cortex, all four females injected with oestrin showed, in their adrenals, a well-developed outer reticular sub-zone. Of the four females injected with testis hormone, three had a fairly conspicuous 'reticularis A' in their adrenals, while one had none. Whether the effect, in the three animals which showed positive results, was due to the male hormone contained in the testicular extract or to the oestrogenic fraction whose presence was likewise demonstrated in the particular preparation used, is not known.

No effect of sex hormone administration, in either male or female, on the adrenal medulla could be demonstrated.

c. Castration

The effects of castration of some duration (of at least 1 month) on the adrenal cortex were identical in both sexes. They expressed themselves chiefly in the manifestation of intensive cell destruction in the reticularis, as well as in the complete or nearly complete suppression of 'reticularis A.' The most typical 'castration picture' in the adrenal cortex consisted of: a wholesale destruction of the innermost rows

TABLE 6
Cortex-medulla ratios in the adrenals of experimental animals

ANIMAL	SACRIFICED DATE	TREATMENT	C	M	C/M
♂ 741	12/ 3/33	Untreated December control	12,042	2067	5.9: 1
♂ 54	1/ 5/32	Pituitary hebin, 1½ cc. daily, 19 days	37,486	2808	13.3: 1
♂ 35	12/12/31	Pregnancy urine, 2 cc. daily, 40 days	26,466	2626	10.1: 1
♂ 358	10/28/32	Antuitrin S, 50 r.u. daily, 19 days	25,375	2858	8.9: 1
♂ 21	10/31/31	+ 100 r.u. daily, 2 days Testis hormone, 12½ bird units daily, 20 days	13,856	2511	5.5: 1
♀ 268	11/21/32	Untreated November control	11,699	2263	5.2: 1
♀ 47	1/ 5/32	Pituitary hebin, 1½ cc. daily, 20 days	53,891	2873	18.8: 1
♀ 194	8/ 1/32	Sheep pituitary suspension, 1 cc. daily, 11 days	22,150	1765	12.5: 1
♀ 192	8/ 1/32	Ovariectomy + sheep pituitary suspension, 1 cc. daily, 11 days	26,880	2194	12.3: 1
♀ 49	1/16/32	Pregnancy urine, 3 cc. daily, 10 days	43,953	3005	14.6: 1
♀ 280	12/12/32	Antuitrin S, 50 rat units daily, 7 days	21,956	1894	11.6: 1
♀ 25	11/18/31	Oestrin, 12½ rat units daily, 19 days	19,236	1795	10.7: 1

of reticularis cells and their replacement by a fibrous ring surrounding the medulla; degeneration foci distributed throughout the reticular layer in the form of 'green cells' ('giant cells'); a total absence of 'reticularis A' at all seasons of the year. The 'giant cells' are designated by the writer as 'green cells' on the basis of the numerous fine, highly refractile granules with which these cells are packed and which stain a brilliant green with Mallory. These granules are probably composed of pigment whose intense yellow blends with the anilin blue of the Mallory stain to produce their

characteristic green hue. The 'green cells' become—in size, number, and mode of staining—a sharply outstanding element in the reticularis of castrates (fig. 7). Whereas, in the adrenals of untreated animals, these cells are rarely present and never conspicuous, in the corresponding glands of castrates they often comprise each an area normally occupied by at least half a dozen cells, and their number may exceed ten per section 4μ in thickness. Each of these formations often contains remnants of as many as three nuclei, but may also be seen—apparently at a more advanced stage—lacking any nuclear remains whatever.

No consistent change in the quantity of lipoids in the adrenal cortex was observed following castration. Similarly, no response was evident in the medulla.

DISCUSSION

The present investigation comprises seasonal observations and experimental studies involving some 350 males and over 300 females. Its results enable the writer to add several suggestive facts to the voluminous and constantly growing body of data regarding a definite functional relationship between the adrenal cortex, on one hand, and the gonads, on the other. For lack of space, the immense literature on the subject will not be reviewed here. Suffice to say that evidence has been forthcoming from three chief sources: clinical facts, experimental results, and normal histology and physiology. The clinical evidence is excellently reviewed by Glynn ('12 and '21) and summarized in a later publication by Goldzieher ('29). It consists chiefly of the genital syndrome accompanying hypernephromata (although the relationship between hypernephromata and the adrenal cortex has not been, as yet, critically demonstrated) as well as of some regressive changes in the reproductive system associated with adreno-cortical atrophy or hypoplasia. Some experimental evidence is reviewed in the introduction to the present report. This evidence is still contradictory, but, with improvement in technique, we shall, no doubt, obtain a greater agreement also in

this field of research. As for observations on the normal morphology of the adrenal cortex in relation to sexual phenomena, several important investigations, notably those of Kolmer ('12) on the guinea pig and Tamura ('26), Masui and Tamura ('26), Deanesly ('28), and Howard-Miller ('27) on the mouse, suggest a definite correlation between the adrenal cortex and the gonads. Just how essential this relationship is, we are still unable to say; in a more recent publication by Howard ('30), she is inclined to minimize the significance of the interaction in question. The fact, however, that such relationship does exist and that the main changes in the adrenal cortex associated with various phases of sexual activity are localized in the reticular zone, is in complete accord with the results of the present work. The study of the adrenal cycle in *Citellus tridecemlineatus* also offers definite proof against the view held by some of the older histologists and apparently still maintained by Tamura ('26) that every zone of the adrenal cortex has an independent origin as well as an independent course of development. This investigation reveals, on the contrary, that in the matter of zonal differentiation and zonal equilibrium in the cortex of the adrenal gland we deal with a highly dynamic situation controlled and regulated largely, if not wholly, by factors endocrine in nature.

SUMMARY AND CONCLUSIONS

A definite interrelationship exists, in the thirteen-lined ground squirrel, between the adrenal cortex and the reproductive cycle. Thus, a significant increase in adrenal size and weight traceable to cortical hypertrophy takes place in the adrenals of both male and female ground squirrels during the breeding period or as a result of artificial stimulation of the gonads and reproductive accessories at the season of sexual inactivity. Histologically, this cortical hypertrophy is associated mainly with the expansion of the reticular zone and the differentiation, within it, of a characteristic, highly developed outer sub-zone termed 'reticularis A' and described

fully. Castration of at least 1 month's duration leads, in the adrenal of either sex, to degenerative changes in the zona reticularis.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 A central section through the adrenal of a normal, untreated female sacrificed in December. A typical winter gland. Bouin; Krichesky-Mallory. $\times 28\frac{3}{4}$.

2 A central section through the adrenal of a female in mid-pregnancy, sacrificed in May. A typical spring gland. Note the middle cortical layer of large cells with dark central portions and light peripheries ('reticularis A'). Bouin; Krichesky-Mallory. Same magnification as in figure 1 ($\times 28\frac{3}{4}$). Compare the size of both sections.

3 Portion of the section represented in figure 1 under higher magnification, showing the entire thickness of the cortex. Note the wide fasciculate zone and the total absence of 'reticularis A.' $\times 170$.

4 Portion of the section represented in figure 2 under higher magnification, showing the entire thickness of the cortex. Note the wide, conspicuous 'reticularis A.' $\times 105$.

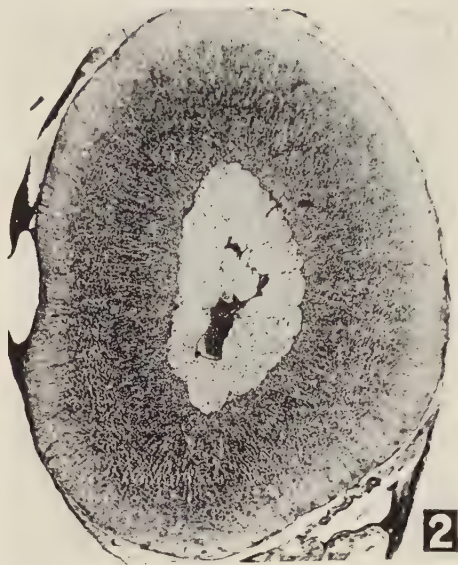
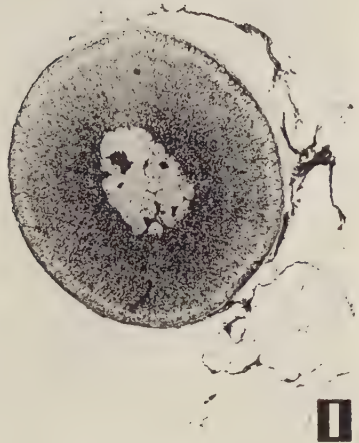


PLATE 2

EXPLANATION OF FIGURES

5 Portion of 'reticularis A' from the section represented in figures 2 and 4, greatly magnified. Note the dense pigment-like material surrounding the nucleus, and the perinuclear and peripheral rings. $\times 1025$.

6 Portion of a section through the adrenal of male no. 35 treated with pregnancy urine (two daily injections of 1 cc. each for 20 days). Note the elimination of the fasciculate zone by the enormously expanded 'reticularis A' and the beginning transformation of the glomerulosa in the 'reticularis A' direction. Bouin; Krichesky-Mallory. $\times 285$.

7 A greatly magnified portion of the 'reticularis B' near the medullary border (which appears at lower left) showing cell degeneration following bilateral castration. Female no. 205, spayed for $2\frac{1}{2}$ months. Note the 'green cells' (giant cells) with pyenotic nuclei. Bouin; Krichesky-Mallory. $\times 1025$.

